Determination of Sulfobetaine Amphoteric Surfactants by Reverse Phase High Performance Liquid Chromatography

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We have developed a method for the direct analysis of sulfobetaine amphoteric surfactants by reverse phase HPLC using methanol/water 80/20 (v/v) as the mobile phase. Elution order correlated with the surface properties of the solutes. This method for determination of (2-hydroxy-3-sulfopropyl) and (sulfopropyl) dimethyl (alkyl) ammonium inner salts was more reliable than indirect determinations of reactants. The accuracy for analysis of sulfobetaines in the reaction mixture was estimated to be 3%. The development of this method expedited studies of reaction conditions so that optimum yields of lime soap dispersants could be obtained. Commercial samples of tallow- and coconut oil-derived amido sulfobetaines were analyzed.

Fat-derived sulfobetaines such as sulfopropylated long chain alkyl quaternary ammonium compounds have been reported elsewhere to be useful as lime soap dispersing agents (*1–3*). However, research studies on such materials have been severely hampered by the lack of a reliable direct method of analysis. Gas chromatographic methods are not applicable because the materials are essentially nonvolatile and cannot be readily derivatized. Reverse phase high performance liquid chromatographic separation (HPLC) and analysis of fatty derivatives such a methyl and phenacyl esters (*4, 5*), methoxy anilides (*6*), or fatty alcohol acetates (*7*) have been reported. Specifically, methanol–water and acetonitrile–water mobile phases have been used for the separation of such nonionized solutes by several workers (*4–7*). The mechanism of liquid chromatographic separations considering surface phenomena were studied by Albertsson (*8*), Guiochon (*9*), and Horvath (*10, 11*). It was of interest to determine in the present study whether the separation mechanism of amphoteric surfactants also involved interfacial phenomena.

The amphoteric surfactants subjected to HPLC separation in the present study were obtained via two different synthetic routes which have been described in detail elsewhere (*2, 3*). They are represented by the following two schemes:

$$ZN(CH_3)_2 + ClCH_2CHOHCH_2SO_3Na \rightarrow ZN^+(CH_3)_2CH_2CHOHCH_2SO_3^- + NaCl \quad (I)$$

and

$$ZN^+(CH_3)_2CH_2CH{=}CH_2\cdot Cl^- + NaHSO_3 \rightarrow ZN^+(CH_3)_2CH_2CH(SO_3^-)CH_2 + NaCl \quad (II)$$

where Z is either a long chain alkyl group or a fatty acid derived long chain amido group of the structure $RCONHCH_2CH_2CH_2-$. The position of the sulfo group of scheme II is not certain, and the resulting amphoteric surfactants are thought to be a mixture of the 2- and 3-sulfopropylated quaternary ammonium compounds.

EXPERIMENTAL

HPLC Apparatus and Operating Conditions. Chromatographic separation of the amphoteric compounds was carried out with the aid of a pneumatically operated pumping system (du Pont 820) fitted with a loop injector (Waters Associates U6K) with a μ-Bondapak-C_{18} column (Waters Associates). A refractometer (Waters Associates Model R-401) was used as a detector. The flow rate was maintained at 1 mL/min. The mobile phase was 80/20 methanol/water.

The test surfactant samples were diluted with 80/20 methanol/water (v/v) or with pure methanol to bring the solvent to this ratio in preparation for HPLC analysis. A 200-μL sample containing up to 1.0 mg pure amphoteric or mixtures of amphoterics was then injected. Standard curves relating peak height to concentration were prepared with the aid of sulfobetaines prepared previously (*1, 2*). Degree of purity for homologous mixtures of sulfobetaines was found by relating the areas of chromatographic peaks run before and after spiking with a known weight of a pure individual amphoteric homolog.

Materials. *N,N*-dimethyldodecylamine (Armeen DM 12D) and *N,N*-dimethylhexadecylamine (Armeen DM 16D), obtained

from Armak Chemical Division, Akzona Inc. (Chicago, Ill.), were better than 99% pure by GLC. The methyl esters of fatty acids were prepared from the appropriate natural products by standard techniques and their purity was determined to be >98% by GLC. The synthesis of sodium 3-chloro-2-hydroxy-1-propanesulfonate and the procedures for the preparation of

$$RN^+(CH_3)_2CH_2CHOHCH_2SO_3^- \text{ and } RN^+(CH_3)_2CH_2CH(SO_3^-)CH_2$$

are described by Parris et al. (*2*). The amido compounds

$$RCONHCH_2CH_2CH_2N^+(CH_3)_2CH_2CHOHCH_2SO_3^- \text{ and}$$

$$RCONHCH_2CH_2CH_2N^+(CH_3)_2CH_2CH(SO_3^-)CH_2$$

were prepared in accordance with the procedure of Parris et al. (*3*).

Pilot plant samples of the amido compounds $RCONHCH_2CH_2CH_2N^+(CH_3)_2CH_2CHOHCH_2SO_3^-$ prepared from coco and tallow fatty acids, respectively, were obtained through the courtesy of the Ashland Chemical Co. (Columbus, Ohio). A 1- mg sample of these two amido compounds was separated by HPLC as shown in Figure 1 (attenuation 16X). The capacity factors for the individual homologues separated were calculated with the use of retention volumes from the chromatograms (Figure 1) and plotted vs. chain length.

Physical Measurements. The amphoteric surfactants of this study were dissolved in a blend of methanol/water 80/20 (v/v), whereas the methyl esters of fatty acids were dissolved in a blend of methanol/water 85/15 (v/v). All physical measurements and HPLC determinations were carried out with the test materials dissolved in the above solvents. Hexane was used to simulate the chromatographic nonpolar stationary phase.

Interfacial tension measurements were carried out with hexane and solutions of test material, at various concentrations, in the appropriate aqueous methanol mixture with the aid of a Du Noüy tensiometer equipped with a 6.0-cm platinum ring.

Distribution coefficients were determined by carrying out batch equilibrations of solutes between hexane and aqueous methanol using previously described methods (*12*).

Monitoring of Sulfobetaine Formation. The following two types of reactions were monitored by HPLC.

(I) *N,N*-dimethyldodecylamine (4.27 g, 0.020 mol) was dissolved in 48 mL methanol followed by the addition of sodium 3-chloro-2-hydroxy-1-propanesulfonate (the addition product of sodium bisulfite to epichlorohydrin) (3.03 g, 0.020 mol) and sodium carbonate (0.42 g, 0.004 mol) dissolved in 12 mL water. The solution was placed in a reaction flask heated to 65 °C with constant temperature bath. The concentration of the reaction mixture was 0.30 M with respect to reactants and contained 20 mol % sodium carbonate. Samples for HPLC analysis were withdrawn at roughly 1-h intervals and % yield of amphoteric surfactant was determined as a function of reaction time as shown in Table III.

The same synthetic procedure was repeated at a concentration of 0.10 M with and without 20 mol % of sodium carbonate. The results are also shown in Table III.

(II) To (allyl) dimethyl (hexadecyl) ammonium chloride (*2*) (13.84 g, 0.040 mol) dissolved in 20 mL methanol was added sodium bisulfite (5.00 g, 0.048 mol) in 20 mL H_2O and the solution was kept at 22 °C. *tert*-Butylperbenzoate (0.194 g, 0.001 mol) was added immediately after mixing to initiate the reaction. The initial concentration of the allyl quaternary compound in the reaction mixture was 0.73 M.

Samples were withdrawn at periodic intervals. The bisulfite content was determined iodimetrically and is discussed later with reference to Figure 2. The methanol/water ratio of the samples had to be adjusted from 50/50 to 80/20 (v/v) by addition of methanol prior to the HPLC determination of sulfobetaine content.

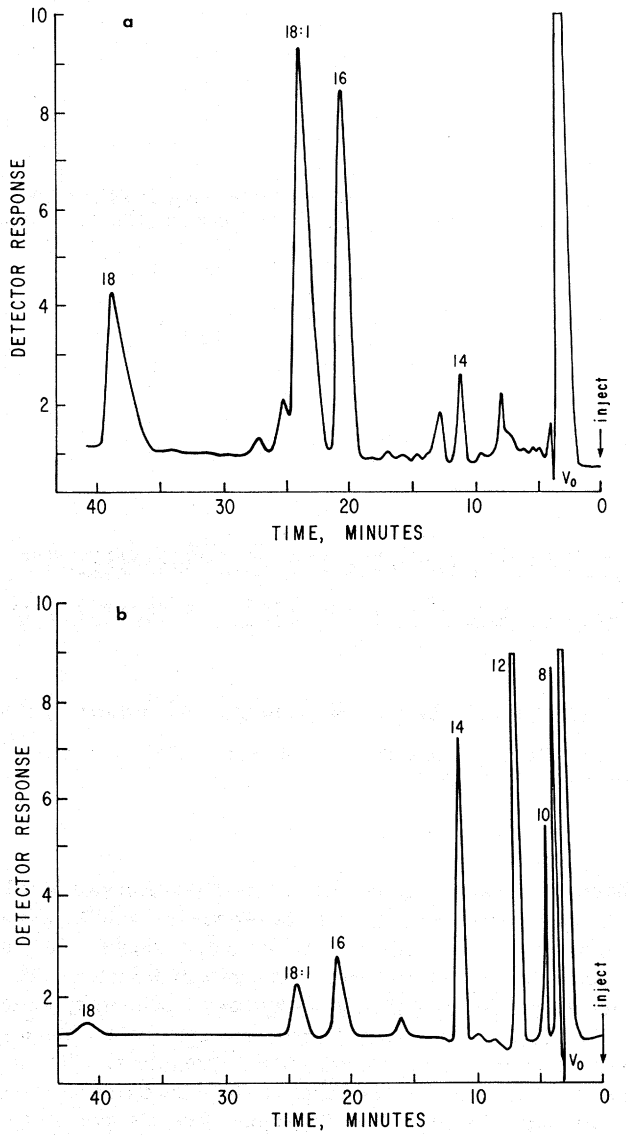

Figure 1. (a) Chromatogram of tallow-derived sulfobetaine. Tal–$CONHC_3H_6N^+(CH_3)_2CH_2CH(OH)CH_2SO_3^-$. (b) Chromatogram of coconut oil-derived sulfobetaine. Coco–$CONHC_3H_6N^+(CH_3)_2CH_2CH(OH)CH_2SO_3^-$

RESULTS AND DISCUSSION

Reverse phase HPLC proved to be a satisfactory method for the separation and identification of several individual homologues of fat-derived sulfobetaines. A refractive index detector was used in this study, since the absence of a suitable chromophore in the test compounds precludes the use of a UV detector. The behavior of these amphoteric surfactants on the HPLC column appeared to differ markedly from that of a homologous series of fatty acid methyl esters. Capacity factors k', calculated from HPLC chromatograms of the methyl esters correlate roughly with distribution coefficients (K) determined in bulk phase equilibrium experiments using hexane to simulte the stationary phase (Table I). The distribution coefficients of the sulfobetaines, $RN^+(CH_3)_2CH_2CH_2CH_2SO_3^-$, on the other hand do not correlate with elution order. The observed capacity factors were much larger than those predicted by Equation 1 using the batch distribution coefficients and considering the fact that the same column was used for both esters and amphoterics, so that V_s/V_m may be considered the same for both series. These relationships are given by:

$$k' = (V_R - V_m)/V_m = KV_s/V_m \quad (1)$$

where V_s is the volume of the stationary phase within the

Table I. Properties of Solutes in Model and Chromatographic Systems

R[a]	Capacity[b] factor (k')	Distribution[c] coefficient (K)	Interfacial[d] tension (dyne/cm)
$RN^+(CH_3)_2C_3H_6SO_3^-$			
Blank			7.5
C_{12}	1.1	4×10^{-3}	4.6
C_{14}	2.4	6×10^{-4}	2.4
C_{16}	5.1	1×10^{-3}	0.1
RCO_2CH_3			
Blank			6.4
C_{16}	6.6	58	5.1
$C_{18:1}$	7.3	71	4.6
C_{18}	12.4	154	4.9

[a] Fatty acid portion. [b] Determined from chromatogram (Equation 1). [c] Determined in equilibrium experiments with model systems (hexane—80/20 methanol/water (v/v) for sulfobetaines, 85/15 methanol/water (v/v) for esters). Precision was ±5% for esters, ±10% for sulfobetaines. [d] At same solute concentration (1.3%).

column, V_R is retention volume of the solute, V_m is the volume of the mobile phase, and K is the distribution coefficient. The interactions between solute, mobile phase, and bound-monolayer stationary phases where the interacting moieties have fewer degrees of motion, differ from those in bulk systems. However, as evidenced by the esters, trends in K values determined in model two-phase systems may be similar to those determined in chromatography. Such similarity was not found for the sulfobetaines.

Since the successful separation of these sulfobetaines by HPLC obviously was unrelated to the bulk distribution coefficients, it was surmised that surface activity contributed significantly to the elution order. Interfacial tension measurements of hexane–aqueous methanol systems containing homologues of $RN^+(CH_3)_2CH_2CH_2CH_2SO_3^-$ as well as of those containing methyl esters are given in Table I. The esters possess essentially no surface activity, whereas the surface activity of the sulfobetaines increases dramatically (i.e., interfacial tension decreases) as the chain length increases. Several researchers have discussed retention mechanisms using models in which surface or interfacial tensions are important contributors (*8–11*). All of the models predict trends in retention similar to those in this study. However, more research is needed to explore the quantitative differences observed and the application of these approaches to surface active compounds.

The differences in surface activity between members of a longer series of sulfobetaine homologues are large enough to effect chromatographic separation. A straight line was obtained when logarithm of capacity factor was plotted as a function of chainlength of R in a homologous series of $RCONHCH_2CH_2CH_2N^+(CH_3)_2CH_2CH(OH)CH_2SO_3^-$.

Chromatograms of commercial samples of a tallow-derived sulfobetaine and a coconut oil-derived sulfobetaine show that good separation of complex mixtures of these surfactants can be achieved (Figure 1). The slight differences in retention times of common components in the chromatograms are due to changes in the column which took place after repeated use over a long time span. The peaks for the saturated homologues were identified by comparison with the retention volumes for the purified homologous sulfobetaines (*2*). Although not identified directly, it is assumed that the peak following the C_{14} compound in Figure 1b is $C_{18:2}$. Comparison of such chromatograms before and after spiking with a known amount of a single solute permits quantification of the amount of total amphoteric in commercial lime soap dispersants. Total area varied by <4%, depending on whether a C_{14} or C_{18} spike was used. Thus while the solutes differ in refractive index, a direct

Table II. Fatty Acid Distribution of Coconut Oil- and Tallow-Derived Sulfobetaines of the Structure $RCONHC_3H_6N^+(CH_3)_2CH_2CH(OH)CH_2SO_3^-$

Fatty acid	Percent homologue in: Tallow-derived sulfobetaines	Tallow (*12*)	Coconut oil-derived sulfobetaines	Coconut oil (*12*)
C_8			12	5-9
C_{10}			8	6-10
C_{12}			49	44-52
C_{14}	4	2-8	15	13-19
$C_{18:2}$	3	1-5	1	0-2.5
C_{16}	31	24-37	8	8-11
$C_{18:1}$	41	40-50	5	5-8
C_{18}	21	14-29	2	1-3

Table III. Effect of Conditions on Reaction I

Time, h	Percent reaction: 0.30 M Reactants, 0.06 M Na_2CO_3	0.10 M Reactants, 0.02 M Na_2CO_3	0.10 M Reactants
0.5	7	...	...
1.5	24	7	2
2.5	41	13	6
7.5	90	44	22

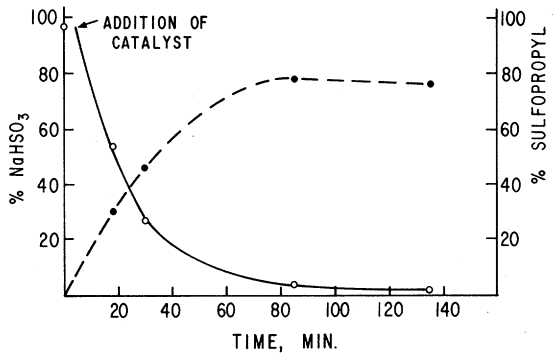

Figure 2. Monitoring of reaction II. (●) % sulfopropylated amphoteric surfactant by HPLC. (O) % $NaHSO_3$ by iodimetric titration

and good estimation of the purity of commercial preparations can be obtained.

The composition of the two commercial samples is shown in Table II. The values obtained are within the range for tallow and coconut oil in a standard reference work (*13*).

On the strength of the above chromatographic separation by HPLC, this technique was applied also to process studies of the synthesis of two types of sulfobetaines. In the process study described elsewhere (*3*), it was pointed out that the formation of the sulfobetaines could not be monitored by conventional means; however we have now shown that monitoring of the sulfobetaine could be successfully carried out by HPLC. Table III shows the rate of formation of the surfactant $C_{12}H_{25}N^+(CH_3)_2CH_2CHOHCH_2SO_3^-$ according to reaction scheme I above under three different conditions. Presence of carbonate buffer and higher concentration of reactants clearly accelerate the reaction.

Bisulfite addition to an alkyl dimethyl allyl ammonium chloride according to scheme II is shown graphically in Figure 2. Here the rate of formation of sulfobetaine is monitored by HPLC while the rate of disappearance of bisulfite is determined by iodimetric titration. Whereas disappearance of bisulfite had been used previously (*3*) as a criterion of completeness of reaction, the HPLC monitoring curve clearly shows a discrepancy between formation of sulfobetaine and disappearance of bisulfite. At the point where all bisulfite

has been consumed, the reaction is only about 78% complete. Thus iodimetric titration is an unreliable means of following the reaction. The accuracy of analysis for both reactions was ±3% when determined for known reaction mixtures.

The significance of the present research lies in the finding that amphoteric surfactants can now be analyzed directly and nondestructively by HPLC. Since a number of analytical methods for the direct determination of all other types of surfactants have been developed in the past, a major gap in analytical methodology has now been filled.

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